233b Living Radical Photografting - a Versatile Technique for Engineering Biofunctional Surfaces

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Our long-term research goal is the design, fabrication, and application of synthetic biomaterials that induce specific biological responses in vitro or in vivo. It has been shown in the literature that cells respond to a wide variety of external stimuli when presented in a gradient fashion. Chemotaxis, haptotaxis, galvanotaxis, and durotaxis are all forms of directed cell migration in response to soluble chemical factors, substrate-bound adhesion molecules, electric field, and substrate compliance, respectively. The goal of the presented work is the nanometer-scale engineering of interfacial polymer layers with spatially defined topographical, mechanical, and biochemical features resembling the bioactive ligand gradients found at in vivo wound sites. Current synthetic methods used to deposit precise densities of biomolecules onto surfaces in a spatially controlled fashion are difficult to perform, resource intensive, and have limited chemical and biological compatibilities. Here, surface-tethered, biofunctional layers will be achieved via living-radical photopolymerization of specialized initiators covalently attached to silicon and polymeric substrates. This novel approach provides the unique ability to fabricate robust bioactive polymer layers with independent control over ligand density, surface topography, and polymer architecture by readily controlling the location and duration of light exposure.

With intelligent application of our living-radical photografting technique, various distributions of grafted-chain lengths can be readily fabricated across a substrate surface including continuously increasing molecular weight gradients of predetermined slope. Modification of the resulting substrate-bound polymer chains can be carried out using well-known procedures to attach bioactive ligands to the polymers. This versatile functionalization scheme can be used to generate areal gradients in polymer molecular weight and ligand density across substrate surfaces as shown in Figures 1 and 2.

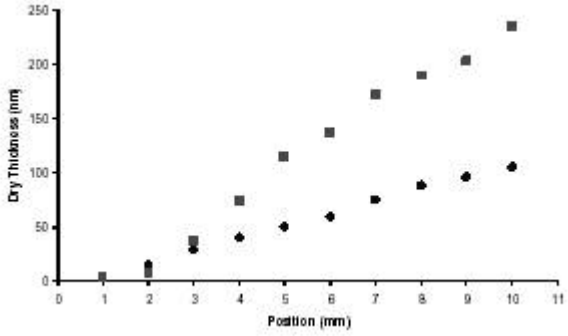

Figure 1. Layer thickness vs. position for surface-attached PMAA (□) and DC-

functionalized PMAA (PMAA-DC) (■) polymerized in a gradient fashion. Exposure conditions: [MAA] = 50% v/v in DI water; Light intensity (365 nm) = 10 mW/cm2. Layer thickness was measured with multiple-angle ellipsometry against a silicon wafer substrate.

In Figure 1, methacrylic acid (MAA) was polymerized in a gradient fashion to yield an interfacial polymer layer ranging in thickness from 0-100 nm on a single silicon substrate. The carboxylic acid groups of the poly(methacrylic acid) (PMAA) chains were then functionalized with a fluorescent probe, dansyl cadaverine (DC). After functionalization, the PMAA chains stretch to greater thicknesses than seen with unfunctionalized chains due to greater steric interactions. As shown in Figure 1, upon functionalization with DC, the dry layer thickness of the functionalized polymer is significantly greater than that of the unfunctionalized PMAA. Further, the slope of the thickness plot for the PMAA-DC polymer is greater than that of the original PMAA indicating an increasing ligand density with position.

The ligand density gradient is verified by the fluorescent image of the PMAA-DC surface. As DC ligand density increases from left to right, an increase in fluorescent intensity is clearly seen in Figure 2.

Figure 2. Fluorescent image of the PMAA-DC film shown in Figure 1 above (top view).

Molecular gradients of cell-interactive ligands have also been created using the living-radical photografting procedure. Immobilization of the integrin binding tri-peptide sequence RGD onto nonadhesive PMAA layers permits cell adhesion in a dose-dependent fashion (data not shown). Further work and cell studies will be conducted in this area. Creating surfaces that present cell adhesion ligands in a tailorable gradient fashion will facilitate the ability to spatially and temporally control cell adhesion and motility across biomaterial substrates used in wound repair therapies.